FUSION OF BLASTOMERES AND NUCLEAR DIVISION WITHOUT CELL-DIVISION IN SOLUTIONS OF NON-ELECTROLYTES.

RALPH S. LILLIE,
UNIVERSITY OF NEBRASKA.

I. INTRODUCTORY.

Blastomeres derived from the cleavage of a single egg-cell have not infrequently been observed to reunite or flow together under various abnormal conditions, producing an apparent reversal of the normal cleavage-process. Thus Dreisch[1] in 1893 observed a refusion of blastomeres in the 4-cell stage of *Echinus* as a result of exposure to temperatures higher than the normal (26 degrees). In 1894 Graf[2] found that compression of *Arbacia* eggs in early stages (16 to 32 cells) also led to a fusion of adjacent cells and even in several instances to a complete reversion from one cleavage stage to the stage immediately preceding (*e. g.*, from 16 cells to 8 cells). In 1896 J. Loeb[3] observed similar phenomena in *Ctenolabrus* eggs deprived of oxygen; he ascribed the effect to a liquefaction of the cell-membrane and a consequent flowing together of the protoplasm of adjacent cells. Phenomena of a somewhat different though closely related order have also been observed from time to time. Ziegler,[4] while studying the cleavage of enucleated blastomeres, observed that these cells frequently underwent only partial division, the cleavage-furrow forming and subsequently disappearing; and similar "abortive attempts at division" have been described by other authors.[5] In such instances the cleavage-process appears at first to proceed normally, but it remains incomplete and the cell resumes its original form when the impulse to division ceases.

[1] Driesch, *Zeitschrift für wissenschaftliche Zoölogie*, 55, 1893, p. 10.
[2] Graf, *Zoölogischer Anzeiger*, 17, 1894, p. 424.
[3] J. Loeb, *Archiv für die gesammte Physiologie*, 62, 1896, p. 249.
[4] Ziegler, *Archiv für Entwicklungsmechanik*, 6, 1898, p. 282.
[5] Cf. Wilson, *Archiv für Entwicklungsmechanik*, 12, 1901, p. 529.

Both of the above classes of phenomena present the following general resemblances: the surface-area of the entire egg first of all undergoes a marked increase; increase of area is accompanied by corresponding alterations in the spherical form of the egg; an increase of surface-extent is thus normal to cleavage and indeed may with justification be regarded as one of its most essential features.[1] The increased surface-area does not in the above instances remain unaltered, or increase still further as normally, but undergoes a secondary and abnormal decrease, seen in the smoothing of the incipient cleavage-furrows or the refusion of the already separate blastomeres. These alterations in surface-area are almost undoubtedly to be ascribed to alterations in the surface-tension of the egg. The primary increase of surface seen in cleavage thus indicates a general lowering of the normal surface-tension; this in the above instances appears to be followed by an increase of surface-tension which leads to the succeeding regressive series of changes.

The problem of cleavage thus resolves itself, partially at least if not entirely, into the problem of the nature of the conditions producing alterations of surface-tension at certain definite regions of the egg-surface. It is believed that the experiments about to be described throw light upon this problem. They have demonstrated that cytoplasmic cleavage is prevented in solutions of non-electrolytes, although nuclear division continues; and also that a strong tendency to fusion makes its appearance in the blastomeres of eggs transferred to such solutions in early cleavage-stages. Since under these conditions the electrolytes normally present in the egg must diffuse outward into the surrounding medium, it is to be inferred that cleavage is closely dependent upon the presence of electrolytes within the egg-protoplasm. The manner in which ions derived from the dissociation of the contained electrolytes may conceivably effect alterations in the surface-tension of the egg will be considered in some detail below.

II. Experimental.

The following experiments were performed during the past summer at Wood's Holl as part of a series on which I was then

[1] Compare Rhumbler: Merkel und Bonnet's Ergebnisse, 8, 1898, p. 605 et seq.

engaged with the aim of determining the influence of the electric current on cell-division in the eggs of *Asterias* and *Arbacia*. Solutions of low conductivity (non-electrolyte solutions with a trace of sea water and isotonic with the latter) were used to insure the passage of the current *through* the eggs. The results of this investigation are incomplete and their publication is deferred for the present. It was found, however, that the simple action of the solutions upon the eggs presented certain interesting peculiarities which form the subject of the following description.

The solutions used were molecular solutions (which are approximately isotonic with sea-water) of urea, glycerine, and cane-sugar, especially urea, which has the least injurious action on the eggs.

In these solutions neither starfish nor sea-urchin eggs are capable of cleavage. Eggs transferred to m-urea-solution shortly after fertilization remain living for several hours and nuclear division continues, although more slowly than under normal conditions. No complete cleavages occur, although many eggs, especially those of *Asterias*, assume irregular or amœboid forms. In *Arbacia* a partial constriction may appear at the equator of the egg if the transfer is made shortly before the time of the first cleavage, but in the majority of instances no such signs of incipient cleavage appear.

The following is a record of a typical experiment:

July 12. *Arbacia* eggs, fertilized at 10:27 A. M., were washed with m-urea and transferred to 100 c.c. of the same solution at 11:10 A. M. At 11:35 the control eggs in the sea-water were beginning to segment; in the urea-solutions no signs of segmentation were visible, but a few eggs had become somewhat amœboid in outline. At 11:55 a larger proportion of urea-eggs were amœboid; a certain number were dumb-bell-shaped with a clear area (nucleus) in each enlargement, the division into two halves remaining incomplete. The great majority of eggs showed no such signs of incipient division. At this time the control eggs were in the 2- and 4-cell stages.

Urea-eggs of this series were preserved in picro-acetic and sublimate-acetic fixing fluids,[1] at stages corresponding to the 2-, 4-, 8- and 32-cell of the control. Subsequent examination of stained preparations showed that nuclear division had proceeded in the urea-solutions although cytoplasmic division had been en-

[1] Boveri's picro-acetic and saturated aqueous mercuric chloride with 2 per cent. glacial acetic acid.

tirely prevented, as above described. In other words, the withdrawal of the electrolytes from the egg in some way prevents the transmission of the division-impulse from the nucleus (which apparently initiates the same) to the body of the cell.

A similar effect has been observed by J. Loeb[1] and Norman[2] to follow the withdrawal of water from the egg by means of sea-water concentrated by the addition of sodium chloride or magnesium chloride; other instances of nuclear division without cell division have been recorded by various authors (Boveri, Chabry, Driesch, Roux).[3] The fact that this phenomenon is seen in solutions of non-electrolytes is of peculiar interest as indicating the importance of the part played in cell-division by the ions present in the cytoplasm. Nuclear division, on the other hand, is apparently independent of the presence of such ions.

Not only is cleavage prevented by transfer to non-electrolyte solutions, but a strong tendency to fusion manifests itself in the blastomeres of eggs transferred during early cleavage stages. This fusion takes place most readily with starfish eggs and in solutions of urea; glycerine- and sugar-solutions are relatively injurious to the eggs and are accordingly less favorable.

The following record will illustrate:

June 27, 1902. Starfish eggs, fertilized at 12:25 P. M., were transferred at 2:15 P. M. while in the 2-, 4-, and 8-cell stages to m-urea-solution. On examination at 3:30 the blastomeres were found in many instances to be completely re-fused, the eggs having apparently reverted to the original unicellular condition. Such eggs form rounded masses of protoplasm each containing several nuclei (which do not fuse) and exhibiting in many cases irregular amœboid projections.

With eggs of more advanced stages (16- and 32-cell) fusion is rarely so complete as in earlier stages. Adjacent blastomeres, however, gradually flow together, and the resulting compound blastomeres, at first hour-glass-shaped, tend to round off, although slowly and as a rule incompletely. Fig. 1 is a representation of an egg with partially fused adjacent blastomeres of the 8-cell

[1] Loeb, J. *Archiv für Entwicklungsmechanik*, Bd. 2, 1896, p. 298.

[2] Norman, *ibid.*, Bd. 3, 1897, p. 106.

[3] For a summary of the observations of these authors cf. Korschelt and Heider, Vergleichende Entwicklungsgeschichte, allgemeiner Theil, erste Lieferung, Jena, 1902, p. 215.

stage. This egg presents an unusually regular appearance; typically the fusions follow no definite order and from the 8-cell stage complexes are more frequently obtained that consist of three, four or five more or less rounded and in part compound blastomeres of unequal size, the entire group remaining enclosed within the egg membrane. Contiguity seems to be the chief condition that determines which of the blastomeres undergo fusion; there is no indication that sister blastomeres reunite more readiiy than cells of different parentage; the fusions take place also after different intervals and with varying degrees of completeness. Thus a strict reversal of cleavage in the sense that the egg reverts to the stage immediately preceding (from 16 to 8 cells, etc.) is of exceptional occurrence. It is of interest to note that fusion is typically preceded by the assumption of a perfectly rounded or spherical form by each cell. The flattening at the surfaces of apposition is thus replaced by a curvature indicative of increased surface-tension (Fig. 2).

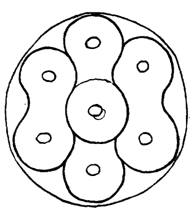

FIG. 1. Example of regular fusion of blastomeres of 8-celled stage. Urea solution. (*Asterias*).

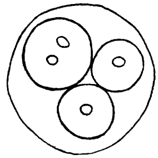

FIG. 2. Rounding of blastomeres in fusion-product of 4-celled stage. Urea-solution (*Asterias*).

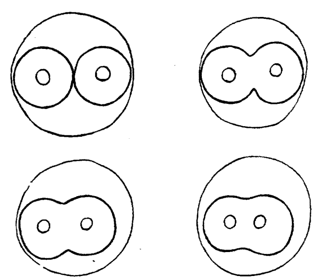

FIG. 3. Different stages of fusion of 2-cell stage in m-glycerine solution (*Asterias*).

In m-glycerine solutions fusions also occur, although in relatively fewer instances. Glycerine is specifically injurious to a far higher degree than urea, and swelling and granular disintegration early appear in many eggs. Different stages of fusion of blastomeres of the 2-cell stage are represented in Fig. 3. Frequently the separate blastomeres merely become rounded without undergoing fusion.

In m-cane-sugar solutions the tendency towards fusion is even less, in consequence apparently of the extreme viscidity and density of the solution and its tendency to withdraw water from the

egg. Actual fusion does not occur in this solution ; the cells become perfectly spherical, touching only at points of their surfaces — indicating an increase of surface-tension — but without fusing.

In sea-urchin eggs similar phenomena are observed. In general fusion takes place more slowly and less completely in these eggs than in those of *Asterias*. *Arbacia* eggs placed in m-urea solution during the 2- and 4-cell stages exhibit fusion in a large proportion of instances ; within thirty minutes after transfer all stages ranging through various dumb-bell-shaped compounds to completely rounded fusion-products are usually seen. Fusions occur also in later stages (16- and 32-cells) in the same manner as in *Asterias*. The blastomeres exhibit a similar tendency to the adoption of a spherical shape before fusion, indicating a preliminary increase of surface-tension.

Fusion occurs also in m-glycerine solutions ; separate blastomeres gradually assume the rounded form, and in a few instances they fuse, but fusion is slower and less complete than in urea-solutions. The glycerine acts destructively on these eggs as on those of *Asterias*, leading before long to a swelling and granular disintegration.

III. Theoretical.

From the above experiments the conclusion may be drawn that cleavage, as well as the maintenance of the cleaved condition, is dependent upon the presence of electrolytes in the cytoplasm. The activity of these electrolytes must be regarded as due to the ions into which they dissociate. The exact nature of the rôle played by the ions is at present largely a matter of conjecture ; but it is reasonable to infer that here, as in other relations, they act chiefly by virtue of the electrical charges which they carry. If this is admitted the following possibilities present themselves for consideration.

If the essential feature of cleavage is an enlargement of the egg-surface due to diminution of surface-tension over certain areas, the possibility at once suggests itself that this alteration of tension may be dependent upon certain known electrical influences,—to be more explicit, upon the appearance of a difference of electrical potential at the surface of contact between the egg

and the surrounding medium. That the surface-tension between two adjacent immiscible fluids may be so altered has long been known; the researches of Lippmann and Helmholtz have shown that under these conditions the surface-tension is greatest when the potential-difference of the two adjoining phases is zero, and decreases in definite proportion as the potential-difference increases.[1] The mutual repulsion characteristic of electrical charges of like sign produces at the surface of contact a certain expansive tension which opposes the normal surface-tension and diminishes the latter by that degree. The deformation of the charged mercury-drop, the expansion of the charged soap-bubble, are familiar phenomena dependent upon diminution of surface-tension so occasioned. It is thus possible that the surface-enlargement leading to cleavage may be a special instance of the Lippmann-phenomenon; if this is true we see at once the importance of the electrolytes, since the production of a difference of electrical potential between the two adjoining aqueous media (egg and sea water, separated by a semi-permeable membrane) can be accomplished only by a migration of ions.

These ions must, under the conditions, be situated within the egg; the appearance of a charge at the surface of the latter must therefore signify the gathering of a surplus of ions of one sign in the superficial regions of the egg-protoplasm. This implies the presence of a corresponding surplus of oppositely charged ions in the interior of the egg. Hence the presence of a surplus of ions of either sign in the peripheral region must imply the existence of a difference of electrical potential between the surface and the interior of the egg; and if the conditions of cleavage are as we have supposed, evidence of such potential-difference should be found at the time of cleavage. Is there any evidence of the existence of such difference of potential, and, if so, how is the same to be accounted for?

In answer to the first question it may be said at once that such evidence is by no means lacking, though of an indirect kind and hitherto variously interpreted. It is seen, in my opinion, in the typical appearances presented by the astral radiations and

[1] Cf. Ostwald, "Lehrbuch der allgemeinen Chemie," 2d Edition, Vol. II., pp. 920–948.

spindle-fibers of dividing cells. The resemblance of the lines of the mitotic figure to the electrical and magnetic lines of force has, needless to say, long been the subject of frequent comment. Now we know that if such an electrical potential-difference between exterior and interior exists, a condition of electrical strain or tension must also exist, the direction of whose lines of traction must correspond with that of the electrical lines of force. It has also been shown that the direction of the fibrils of coagulum in fixed preparations of albumin-solutions subjected during fixation to mechanical strain coincides closely with the direction of this strain (cf. Hardy,[1] Fischer [2]). We may therefore infer from the direction of the fibrils in fixed preparations of dividing cells that a condition of strain or tension exists between interior and periphery of the cell during mitosis, similar to that which would exist in the presence of an electrical potential-difference of the kind imagined above. This agreement confirms the theory that such potential-difference does actually exist. The tendency of the alveoli in echinoderm eggs to dispose themselves along corresponding lines is similarly to be interpreted as an instance of the tendency possessed by polarizable particles in an electric field to arrange themselves in rows along the lines of force.[3]

It may fairly be claimed therefore that it is no mere assumption to suppose that during cleavage the surface of the egg is charged differently from the interior. If such a surface-charge is present it will infallibly produce a lowering of the surface-tension with corresponding changes in the form of the egg. Cleavage, on the present theory, is the result of such changes: its conditions will be considered below in further detail.

If we grant then that during mitosis the surface of the egg is charged differently from the interior the question arises: how is this difference of potential established and maintained? In other words, what influence directs ions of one sign toward the periphery, the others toward the interior of the egg? This question ap-

[1] Hardy, *Journal of Physiology*, 24, 1899, p. 158.

[2] Fischer, Fixierung, Färbung und Bau des Protoplasmas, Jena, 1899.

[3] For experiments bearing directly on this question, see Gallardo, "Interpretacion Dinamica de la division Cellular," Buenos Ayres, 1902. Reviewed by M. Hartog in *Nature*, Vol. 67, 1902, p. 42.

pears at present susceptible of only partial reply. It is to be noted however that a marked potential-difference *invariably* makes its appearance (as evidenced by the appearance of astral radiations) whenever the nuclear chromatin passes into the highly condensed and chromatic phase, characterized by a high proportion of nucleic acid, which forms perhaps the most typical and constant of the peculiarities of mitosis. Since, chemically considered, this change denotes an increase in the acidity of the chromatic colloids, and since such acidity implies that the colloidal particles are negatively charged,[1] the inference has seemed reasonable that the inequality of distribution of the ions in the cytoplasm is due to the acquisition of a negative charge of high potential by the colloids composing the nuclear chromatin. I have elsewhere[2] called attention to this possibility and have pointed out that the spiral form of the chromatic filament in the prophase, and also the disposition, mode of division, and movements of the chromosomes, are strongly suggestive of the action of electrostatically charged bodies. If the chromatin carries a negative charge the anions will tend to approach the periphery of the egg as a result of the inductive action of this charge, while the kations will be attracted toward the chromatin. The result will be the establishment of a potential-difference within the cytoplasm of the kind indicated above, with a negative charge at the surface of the egg.

There exists experimental evidence of an apparently conclusive kind that the ions of opposite sign present in a solution may be separated by the inductive action of an electrostatically charged body. The original proof of this proposition appears to be furnished by the well-known and frequently-quoted experiment of Ostwald and Nernst.[3] Recently J. Olsen[4] has described experiments which lead to similar conclusions. This author has found that an electrolyte-solution is affected by an electrostatic charge in such a manner that the liquid nearest the charge assumes a charge of opposite sign to that of the charged body employed,

[1] Picton and Linder, *Journal of the Chemical Society*, 1897, LXXI., p. 568. Hardy, *Journal of Physiology*, 24, 1899, p. 288. See also Bredig, "Anorganische Fermente," Leipzig, 1901, p. 15.

[2] *American Journal of Physiology*, 8, 1903, p. 273.

[3] *Zeitschrift für physikalische Chemie*, 3, 1889, p. 120.

[4] *American Journal of Science*, 1902, Vol. XIV., No. 82.

while the remotest portion of the liquid assumes a charge of the same sign as that of the charged body. The liquid, in other words, behaves as if it contained charged bodies free to move (ions) which may be separated by the inductive action of an electrostatic charge, ions of opposite sign moving toward, those of the same sign away from the inducing charge.

In the cell the charged body on the above theory is represented by the chromatin; by the inductive action of its negative charge the anions are repelled toward the periphery of the egg while the kations are attracted toward the nucleus. The center of the astral radiations must on this view represent the region of highest positive potential — the region, that is, in which the kations are most densely aggregated. Exactly why there should be *two* such regions, and why these are situated on opposite sides of the nucleus and at some distance from the surface of the latter are questions of considerable difficulty which cannot be considered in detail in the present paper. It is evident that the conditions of electrostatic equilibrium in such a structure as the cell must be of somewhat complex nature. Later I hope to consider these and other questions in fuller detail; for the present their treatment is deferred.[1]

[1] I may suggest here briefly that the *form* of the cell must influence the distribution of the surface charges; and that it is for this reason that the direction of the spindle-axis bears certain well-defined relations to the principal axis of the cell; these relations have long been known and are formulated in Hertwig's laws of cell-division. If the cell possesses approximately the shape of a prolate ellipsoid — a not infrequent condition — it is to be expected that the repellent action of the internal negative charge will produce a tendency toward aggregation of anions at those regions of the surface-area which are most remote from the central charged body, *i. e.*, which adjoin the long axis of the cell. If the surface negative charge so induced attains sufficient density in these regions there must result a tendency for internally situated kations to be attracted toward the poles of the cell as well as toward the chromatin; kations will therefore tend to collect in each half of the cell in a certain position of equilibrium at which these opposing tendencies are balanced; the position of these regions will vary with variations in the electrical condition of the chromatin, but will typically be somewhere between the chromatin and the extremities of the long axis of the cell. The astral centers represent these regions of highest positive potential; hence they form the centers toward which the electrical lines of force converge. Under the usual conditions they are two in number and adjoin the long axis of the cell. The positions of the two may coincide at their earliest appearance; hence an originally single aster, situated in close proximity to the nucleus, appears to divide into two which recede toward opposite poles of the cell.

The preponderance of negatively charged ions at the surface of the egg must result in a fall of surface-tension, and it is to this that cleavage on the above theory is supposedly due. That alterations of surface-tension do actually accompany a passage of the chromatin into the chromatic phase, even when normal cleavage fails to occur, seems proved by certain recent observations of E. B. Wilson[1] on artificially fertilized eggs of *Toxopneustes*. It was found that a limited number of eggs after having been exposed to the action of the fertilizing solution (mixture of equal parts sea water and 12 per cent. magnesium chloride) do not segment (as do the majority of eggs thus treated) but undergo the following abnormal changes: "the nuclear area gives rise to a single radiation or monaster which never resolves itself into a bipolar figure. Such eggs never properly segment, but pass through regularly alternating phases of nuclear transformation parallel to those of progressively dividing eggs." That is, first the nucleus enters the resting phase and the astral radiations become greatly reduced; then the nuclear membrane again disappears and the astral radiations regain their original prominence; this is again followed by the reconstruction of the resting nucleus and the reduction of the radiations. The above cycle of changes may occur several times in succession in a single egg. At each disappearance of the nuclear membrane a group of granules appears in the clear center of the aster; these are believed to be chromosomes. These bodies progressively multiply by longitudinal division until finally they may become very numerous. The important fact from our present

An interesting incidental result of these conditions is a tendency for any minute electrically negative particles casually present in the cytoplasm to be drawn along the lines of force toward the regions of highest positive potential. Here such particles must gather and remain, and in stained preparations they may present the appearance of prominent deeply staining bodies occupying the astral centers. On the above theory the centrosomes originate in some such manner as this. The affinity exhibited by these bodies for basic or nuclear dyes may be regarded as additional evidence of their acidity and electrical negativity. It should be remarked that other authors have regarded the centrosomes as formed by the aggregation of centripetally moving microsomes (cf. Bürger, *Anatomischer Anzeiger*, 1892, p. 222). On the above theory this migration is due to electrical influences. Later, however, I hope to treat these and related questions in a somewhat less summary manner.

[1] Wilson, *loc. cit.*, pp. 546, 547.

standpoint is that "*during the telophase the egg frequently becomes amœboid*, and may even make an abortive attempt to divide. In the later stages in some cases it may actually divide into a number of irregular masses, only one of which contains a nucleus (Fig. 8, *i*), but which here again completely fuse together." These phenomena are almost unquestionably the result of an alteration of surface-tension; at all events they are precisely what might be expected to occur if such alteration in surface-tension were to take place. It may therefore be regarded as an established fact of observation that during the chromatic nuclear phase certain influences are active which produce alterations in the surface-tension of the egg. That these influences are of an electrical nature may, in view of the above facts and considerations, be regarded as at least highly probable.

The increase in the surface-area of the egg during division may be held to denote a general decrease of surface-tension. But a perfectly uniform decrease of tension over the entire surface could lead to no change of form; this is evident from the fact that fluid droplets of unequal surface-tension alike tend to assume the spherical form when under the sole influence of such surface-tension, provided this is uniform at all portions of the surface. Changes of form in such droplets result when the surface-tension becomes unequal at different regions of the surface. Thus if the tension is lowered over a small circumscribed area (as appears for instance to occur in the egg during the formation of the polar bodies) the fluid tends to flow outward or be pressed outward at that region — the internal pressure being there insufficiently compensated for the retention of the spherical form — and an amœboid projection is the result. Perfectly definite changes of form, such as occur in cleavage, imply a correspondingly definite localization of the areas of lowered surface-tension. In the case of the egg various indications — such, for example, as the preliminary elongation in the direction of the spindle-axis — point to the conclusion that surface-tension is primarily lowered at the two sides of the egg opposite the astral centers. From the position of the asters during the metaphase and telophase it is to be expected on the present theory that the surface negative charge will be densest near the regions adjoining the long axis of the

egg, and that there the surface-tension will accordingly be lowered to the greatest degree. It is also clear that the influence in these regions will increase as the daughter groups of chromosomes approach the poles since the inductive action of the chromatin on the cell-surface must increase as the interval between the two diminishes. The surface-tension at the regions adjoining the astral centers must therefore decrease still further as the daughter groups of chromosomes approach the surface ; in other words, the difference between the surface-tension at the polar and at the equatorial regions of the egg (speaking with reference to the spindle axis) progressively increases as the groups of chromosomes diverge. Eventually the egg is surrounded by an equatorial surface-zone possessing a distinctly higher tension — *i. e.*, a stronger tendency to contract — than the polar surface-areas. The effect is naturally the same as would be produced by the presence of a *constricting band* surrounding the equator ; a "cleavage-furrow" appears which progressively deepens until complete bipartition is effected.[1]

Cleavage, on this theory, is the result of an inequality of surface-tension between polar and equatorial regions of the dividing cell, due to a greater lowering of surface-tension at the poles than at the equator. This diminution of surface-tension is dependent on the ions present in the protoplasm, hence withdrawal of these, as by the use of non-electrolyte-solutions, prevents cleavage, and, by heightening the normal surface-tension, tends to favor fusion of adjacent blastomeres.

Why similar effects should follow withdrawal of water and compression is not clear. It might be suggested that the effect of compression is an instance of the second of Lippmann's laws cited by Ostwald[2] in his "Lehrbuch": if by mechanical means the surface of the fluid (with tension lowered by the electrical double

[1] So early as 1876 Bütschli (*Abhandl. d. Senkenbergschen Naturf. Ges.*, Bd. 10) referred division to changes of surface-tension due to influences emanating from the astral centers. In his recent paper (*Archiv für Entwicklungsmechanik*, 10, 1900) the equatorial constriction is ascribed to a relative increase in the surface-tension of the equatorial zone of the egg. Conklin (*Journal of the Academy of Natural Sciences of Philadelphia*, Second Series, Vol. XII., Part I., pp. 95 et seq.) is in essential agreement with Bütschli so far as regards the immediate origin of the cleavage furrow.

[2] Ostwald, *l. c.*, p. 923 ; see also *ibid.*, p. 929.

layer) is increased, the electrical potential-difference of the surface also alters in such a sense as to resist a continued increase of surface, *i. e.*, surface-tension is increased. The increase of surface due to compression of the eggs — supposing the conditions to be of the above kind — must therefore heighten the normal surface-tension ; it will consequently promote fusion. With regard to the effects of withdrawal of water and raising of temperature I have no suggestions to offer at present. Withdrawal of water would result in a decrease in the number of dissociated ions, but it is doubtful if the effect could be attributed to this cause alone.

IV. Concluding Remarks.

On the view briefly sketched above mitosis is an incidental consequence of the passage of the chromatin into the strongly acid and chromatic phase ; this change involves the acquisition by the chromatin of a negative charge of considerable potential, as a result of whose inductive action there ensues a redistribution of the ions in the cytoplasm with the production of certain differences of electrical potential. To these potential-differences are due the appearance of the astral radiations and the diminution of surface-tension that leads to cleavage.

If this theory is well-founded it is evident that the ultimate determining conditions of mitosis must be sought in the conditions that control the chemical changes in the chromatin, especially those affecting the proportion of nucleic acid in this substance. In the mature egg the passage into the chromatic state normally follows the introduction of a spermatozoön ; but the same change may be artificially induced, as shown by J. Loeb and his collaborators, by withdrawal of water, action of certain electrolytes, or even by mechanical agitation. The change is of a metabolic nature and as such is presumably dependent in large part on the action of ferments. We may assume that the liberation of the enzymes concerned, or their activity when liberated, may be dependent on the presence of certain electrolytes. Further speculation on this problem, however, seems premature at present.

Only the most general conditions of mitosis have been considered above. It is evident that, among other conditions, the

state of permeability of the egg-membrane to ions, and the metabolic changes in the cytoplasm, resulting as these undoubtedly do in the production of ions, must be considered in a complete theory. It should also be borne in mind that potential-differences in the cytoplasm may conceivably originate otherwise than by induction;[1] that this is actually so is indicated by the phenomena of artificial asters or asters in enucleated eggs (cf. Morgan, Ziegler, Wilson). It seems probable however that in mitosis induction plays the chief part in the production of radiations; the formation of the sperm-aster appears to be another instance of the same phenomenon. In either case astral radiations are to be regarded as the visible expression of local differences of electrical potential within the cytoplasm.

[1] The localized production (*e. g.*, as an excretory product) of any electrolyte having ions of decidedly different migration-rates would have this effect; it would be especially marked in the case of acids (*e. g.*, lactic acid) on account of the high speed of migration of the hydrogen ion.